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RESEARCH

# Clinical significance of peripheral T-cell repertoire in head and neck squamous cell carcinoma treated with cetuximab and nivolumab

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## Abstract

Immunotherapy holds significant promise for treating head and neck squamous cell carcinoma (HNSCC), yet responses are limited to a subset of patients. This research investigates whether analyzing the peripheral T-cell receptor (TCR) repertoire could help identify patients who are more likely to benefit from a combination treatment of cetuximab and nivolumab. We report here updated correlative analysis using all samples profiled with deep immunoSEQ assay to study the peripheral TCR repertoires in peripheral blood mononuclear cells from patients enrolled in a phase I/II trial (NCT03370276). TCR repertoires were analyzed in 67 patients. Of these, 64 had available baseline data. Overall, our findings confirm that a more polyclonal peripheral TCR repertoire is associated with improved response to concurrent cetuximab and nivolumab in HNSCC. While the baseline productive Simpson clonality did not reach statistically significant differences in response groups, significant differences were observed within the HPV-negative subgroup and among patients who had received previous ICI therapy. Additionally, the TCR diversity at baseline and early follow-up was associated with overall survival. TRBV/TRBJ gene usage analysis also identified specific gene pairs associated with patient outcomes. Furthermore, our analysis indicates that the TCR clonality patterns are modulated by prior treatment exposures and tumor HPV status, suggesting a cohort expansion within these subgroups for further validation. Together, this study demonstrates the feasibility of leveraging the peripheral T-cell repertoire profiling and clonality dynamics as predictive biomarkers for immunotherapy efficacy in HNSCC.

**Keywords** T-cell repertoire · Head and neck squamous cell carcinoma · Peripheral blood · Nivolumab · Cetuximab · Immunotherapy

## Introduction

Head and neck squamous cell carcinoma (HNSCC), originating in the mucosal epithelium of regions such as the oral cavity, pharynx, and larynx, constitutes approximately 90 percent of all head and neck malignances. The management of the disease remains a significant therapeutic challenge, casting a substantial impact on patient outcomes and the healthcare burden both within the USA and globally [1, 2]. HNSCC can be classified into distinct clinical subtypes according to risk factors such as human papillomavirus (HPV) status and smoking history, with HPV-negative tumors often carrying a poorer prognosis. HNSCC's immunogenic nature opens opportunities for novel therapeutic interventions [3]. Recent advancements in cancer immunotherapy, particularly the use of immune checkpoint inhibitors (ICIs), have demonstrated efficacy in a subset of patients with HNSCC [4–6]. The ICIs, including

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nivolumab and pembrolizumab, have received FDA approval for treatment of patients with recurrent and/or metastatic (R/M) HNSCC. Built on the success of ICI monotherapy, combination approaches such as cetuximab plus nivolumab are actively being investigated through both preclinical and clinical research [7, 8]. Despite these advances, the response rate to ICI therapy remains relatively low in HNSCC. Only 20–30% of patient will derive long-term clinical benefit from anti-PD1 monotherapy or combination therapies [6]. This highlights the critical need for further research to identify biomarkers of clinical benefit and mechanisms that can facilitate appropriate patient selection and more effective combinations. Metrics derived from the peripheral blood T-cell receptor (TCR) repertoire hold potential as valuable biomarkers in this context.

Peripheral blood-based biomarkers for analysis offer compelling advantages over tumor tissue biopsies, as it provides a less invasive approach and allows for multiple and real-time monitoring. It is hypothesized that both tumor-specific and host-specific T-cell clones might undergo clonal expansion and shift as they mount an immune response against cancer cells. The analysis of these T-cell clones provides insight into how the host's immune system is primed in responding to immunotherapy. Therefore, various TCR metrics can be established as potential pre-treatment biomarkers, offering predictive information about a patient's benefit to therapy [9–12]. The continuous monitoring of the diversity and abundance of TCR clonotypes may also inform more adaptive immunotherapies. Furthermore, although very challenging, the identification of specific TCR clonotypes associated with positive treatment responses may guide the design of novel immunotherapies aimed at expanding these beneficial immune cell populations.

In our previous study [13], we investigated the potential of the TCR repertoire in peripheral blood mononuclear cells (PBMC) as a biomarker for predicting the efficacy of concurrent cetuximab and nivolumab therapy in HNSCC. Overall, our results suggested a trend toward favorable patient outcome within the patient group exhibiting a polyclonal TCR repertoire. However, the study also revealed a range of critical challenges in leveraging the peripheral TCR repertoire as a robust immunotherapy biomarker. Firstly, we noted that Adaptive immunoSEQ survey-resolution assay often yielded less reliable diversity metrics and biased estimates of less abundant TCR clonotypes compared to deep-resolution panels. Secondly, the extreme heterogeneity of TCR repertoires among patients posed difficulty in identifying and validating shared clonotypes, e.g., shared in responder patient groups. Thirdly, the scarcity of bioinformatics databases for cancer-specific TCR functional annotation and antigen identification hindered cross-validation analysis and interpretation. Finally, patient heterogeneity in HNSCC, influenced by factors such as HPV and smoking status, further complicated the analysis and interpretation of TCR repertoire data. It is important to note that both HPV and smoking status have recognized impact on the host immune response. These challenges underscore the need for substantial expansion of TCR assays and database specific to this disease, necessitating both increased sequence depth and a broader patient spectrum.

In this paper, motivated by the aforementioned challenges, we report an expanded TCR beta (TCR-B) analysis based on PBMC collected from patients who participated in a phase I/II trial investigating the concurrent use of cetuximab and nivolumab for R/M HNSCC [14, 15]. All TCR sequencing analyses were conducted based on the Adaptive immunoSEQ deep TCR-B assay, in contrast to our previous analysis which was primarily based on the survey-resolution assay. Our primary analytical goal is to examine if there are any differences in the baseline TCR diversity among patient subgroups—categorized by prior ICI therapy, smoking history, and HPV status—in relation to their immunotherapy outcomes. In addition, based on the deep assay TCR profiles from different time points, we aim to investigate the potential prognostic value of these metrics in forecasting long-term patient survival. The TCR repertoire data collected also serves as a valuable resource for future studies, advancing our understanding of immune responses in HNSCC and potentially guiding alternative therapeutic strategies.

## Materials and methods

This expanded cohort study of TCR repertoire enrolled patients from a phase I/II trial involving concurrent cetuximab and nivolumab treatment in patients with R/M HNSCC, registered under NCT03370276 [14, 15]. The trial and correlative analyses received approval from the Scientific Review Committee, the Institutional Review Board at Moffitt Cancer Center, and all other participating sites. PBMC samples from 67 patients were evaluated in this study, of which 41 were part of our preliminary study (using the immunoSEQ survey-level assay) as illustrated in Fig. 1a.

### Specimen collection and processing

The procedures for blood sample collection, PBMC isolation, buffy coat processing, and DNA extraction and quantification were previously described [13]. Briefly, blood samples were collected into either 10-ml $K^2$ EDTA Vacutainer tubes or LBgard Blood Tubes. The PBMCs were isolated from the buffy coats using density-gradient separation and cryopreserved in a freezing media until DNA extraction. DNA extraction was performed using the QIAamp DNA blood mini kit, with concentration and quality assessed using a NanoDrop spectrophotometer and Qubit dsDNA Assay

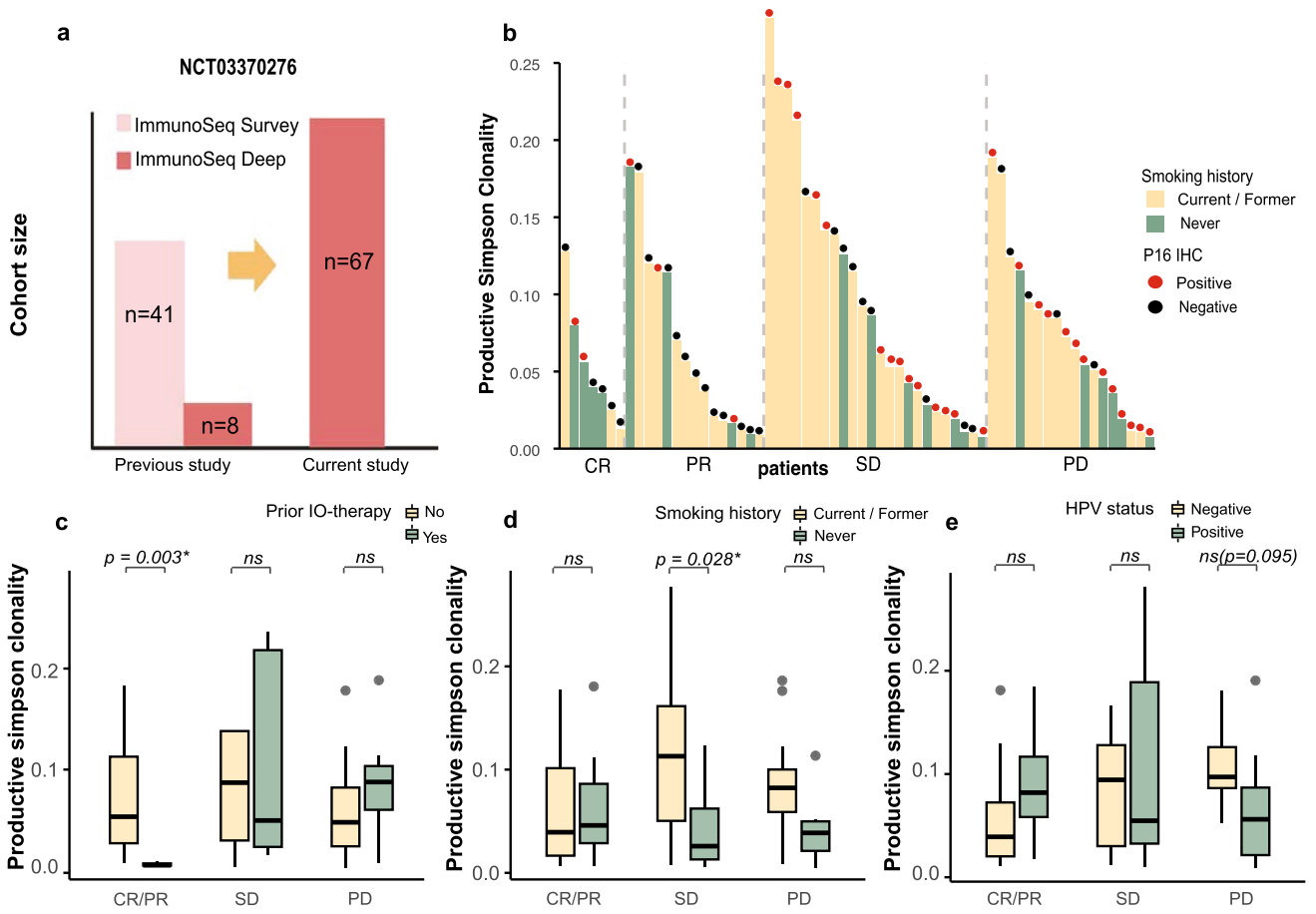


**Fig. 1** Patients with HNSCC and pre-treatment TCR clonality and diversity across patient subgroups. **a** Overview of patient enrollment and sample collection. **b** Simpson clonality distribution across all patients with HNSCC, stratified by treatment response groups (CR, PR, SD, PD). **c**–**e** Productive Simpson clonality index across various patient subgroups categorized by prior IO therapy, smoking history, and HPV status. The two-group comparisons within treatment groups were based on Wilcoxon test. The trend test of TCR diversity pattern across treatment groups is presented in Supplementary Fig. 1

Kits. The specimen collection and DNA isolation were performed by Moffitt Cancer Center Tissue Core.

## TCR sequencing and analysis

The distribution of primary disease sites includes 21 oral cavity, 31 oropharynx, 7 larynx, 3 hypopharynx, and 2 p16-positive unknown primary of head and neck region. Of the cohort, 33 (51.6%) were HPV-positive as determined by p16 immunohistochemistry. Other clinical characteristics are detailed in Supplementary Table 1.

TCR sequencing was performed using deep immunoSEQ assay (immunoSEQ Deep) at the Moffitt Cancer Center's Molecular Genomics Core facility. The TCR repertoire analysis was again conducted using the Adaptive Biotechnologies immunoSEQ human T-cell receptor beta (hsTCR-B) kit. The deep assay requires a greater input DNA amount but offers a more inclusive analysis of T-cell clones, including those present at lower frequencies, thereby enabling a more accurate estimation of various diversity and clonality metrics. If feasible, PBMC samples were collected and analyzed by immunoSEQ at four time points during the treatment: pre-treatment; on-treatment after lead-in cetuximab or after Cycle 1 Day 1 of cetuximab and nivolumab (C1D1); on-treatment after Cycle 4 Day 1 (C4D1); and End of Treatment (EOT).

The generated TCR-B sequence data were processed using the immunoSEQ Analyzer software developed by Adaptive Technologies (available at http://www.immunoseq.com), or alternatively, the data were exported to the R environment for additional exploratory analysis using the immunarch package. In our analysis, productive Simpson clonality was employed as the primary metric for assessing TCR diversity, which is derived from the square root of Simpson's diversity index applied to all productive rearrangements within a patient sample. The Simpson clonality

ranges from 0 to 1, with values approaching 0 indicating higher polyclonality within a sample. Adaptive recommends Simpson clonality as it demonstrates reduced sensitivity to sequence depth discrepancies across experiments, providing a more consistent measure of TCR diversity than metrics such as Shannon Clonality.

### Statistical analysis

A series of statistical analyses were conducted to investigate the associations between TCR diversity, gene usage patterns, and patient clinical outcomes. To analyze clonality metrics between clinical subgroups, we employed the nonparametric Wilcoxon test, facilitating comparisons across patient subgroups and treatment responses—complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). To examine the pattern of TCR diversity among different treatment groups, we utilized the Kruskal–Wallis test and Cuzick's nonparametric trend test, evaluating the groups ordered from CR/PR to PD. Kaplan–Meier survival plots along with the log-rank test were utilized to assess patient survival differences across various TCR diversity groups and at distinct treatment time points. In our study, we extensively analyzed TRBV/TRBJ gene usage patterns as a means to gain deeper insight into the V–J pairing distribution within the PBMC TCR repertoire. The prognostic significance of these gene usage combinations was evaluated and prioritized using the Cox regression model. Additionally, we conducted exploratory data analysis using the immunarch package in R, which allowed for the visualization and further evaluation of TCR diversity and clonality metrics.

## Results

### Pre-treatment TCR clonality and diversity across patient subgroups

Figure 1b illustrates the Simpson Clonality, a key metric recommended by Adaptive Technologies for assessing TCR repertoire diversity, across 64 patients with HNSCC who had evaluable data at baseline. A smaller value of Simpson clonality (closer to 0) indicates greater diversity or polyclonality in the TCR repertoire. Figure 1b shows an overall trend of decreased TCR diversity (increased Simpson Clonality) across CR/PR/SD/PD groups, which is in line with our earlier study based on the survey-level immunoSEQ from 41 patients. Patients with a CR or PR displayed a more polyclonal TCR repertoire relative to those with SD or PD, with the CR group exhibiting a higher degree of polyclonality than the PR group. Interestingly, the analysis revealed that patients in the SD group did not generally exhibit a more polyclonal TCR pattern than those in the PD group. However, when assessed as a continuous variable, the differences of baseline productive Simpson clonality across response groups did not reach statistical significance (Kruskal–Wallis test, $p = 0.35$), as shown in Supplementary Fig. 1a.

We then examined the productive Simpson clonality index across the various patient clinical subgroups, as depicted in Fig. 1c–e. Predominantly, the CR/PR group across all subgroups displayed more polyclonal TCR profiles than the SD and PD groups. This overarching trend aligns with our earlier findings. However, the new analysis also uncovered intriguing patterns within each patient subgroup. Specifically, when stratifying patients by their prior ICI therapy history specifically, those who had previously received ICI therapy exhibited elevated diversity within the CR/PR group, indicated by lower productive Simpson's clonalities ($p = 0.003$), while experiencing decreased diversity in the PD group with higher Simpson's clonalities. When stratifying patients based on smoking history, we observe that current/former smokers have higher Simpson clonality than never smokers in SD and PD groups, with a significant difference in the SD group (Wilcoxon test, $p = 0.028$). Interestingly, when we only compare the scores in current/former smokers (yellow-colored boxplots in Fig. 1d), the trend toward a more polyclonal TCR in the response group became even more pronounced. Lastly, when stratified by HPV status, HPV + patients exhibited lower Simpson clonality in the PD group ($p = 0.095$). Similarly, when examining exclusively on HPV-negative patients (as depicted by the yellow-colored boxplots in Fig. 1e), a more evident trend toward decreased TCR polyclonality was observed moving from the CR/PR group to the PD group with gradually increased Simpson's clonalities. The formal statistical tests, presented in Supplementary Fig. 1, indicate that there is significant difference within both the prior-IO and HPV-negative subgroups (Cuzick's trend test: $p = 0.041$ and $p = 0.045$, respectively).

### The association of temporal TCR metrics with patient survival

In our study, we further conducted an in-depth investigation into the relationship between temporal TCR metrics and long-term patient benefit, specifically patient survival, across four significant time points: T1, pre-treatment; T2, on-treatment after lead-in cetuximab or after C1D1 cetuximab and nivolumab; T3, on-treatment after C4D1; and T4, EOT. However, TCR data were not available at all time points from each patient due to variable responses and patient's ability to return to the clinic for blood collection. This resulted in varying patient sample sizes with 64, 44, 35, and 31, respectively, for these four time points. Undoubtedly, T1 is the most important treatment timepoint for predictive biomarker development because it offers valuable insights

into the patient's immune condition prior to the initiation of the immunotherapeutic interventions. T2 is also considered a critical treatment milestone in this trial because it provides early indications of patient response and immune priming. In contrast to the prior analysis, the PBMC collected at these time points were all examined based on the TCR-B deep-resolution assay.

Figure 2 presents Kaplan–Meier plots illustrating patient overall survival outcomes across the observed time points, stratifying patients into high and low TCR diversity groups. To gain a more comprehensive analysis of the prognostic potential of TCR metrics, we employed three metrics for measuring TCR diversity: productive rearrangement, Shannon index, and Simpson clonality. In the current updated analysis, we noted that the prognostic potential of TCR metrics is more pronounced during the early stages of treatment (T1 and T2). Consistent with our earlier findings, we observed that at T1 (pre-treatment), patients with a more polyclonal TCR profile exhibited a more favorable prognosis, although statistical significance was marginal. Note that, in our previous analysis, the prognostic significance was only assessed based on the survey-resolution data from a limited cohort of 38 patients. The limited sample size also prevented us from exploring additional time points after T1. Of particular interest in the update analysis is T2, representing the initial phase of immunotherapy, where we observed a notable enhancement in the significance of TCR metrics. The separation between high and low TCR diversity groups in terms of survival became more pronounced. In productive rearrangements, *p*-values improved from 0.076 to 0.042, in Shannon index from 0.08 to 0.04, and in Simpson clonality from 0.14 to 0.089. This observation suggests that early immune responses and TCR diversity during the initial treatment phase may indeed hold prognostic significance in

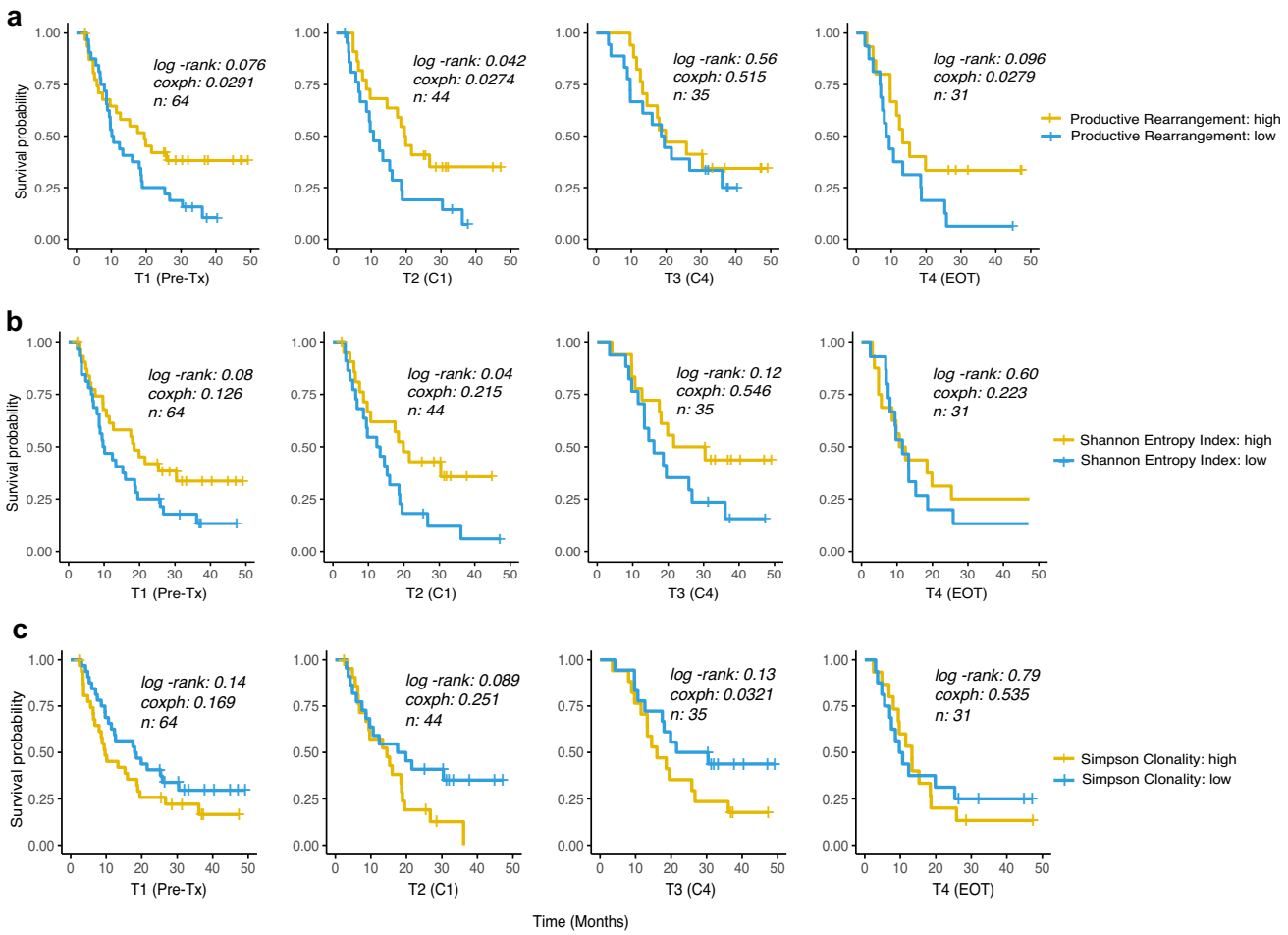


**Fig. 2** The association of temporal TCR metrics with patient overall survival. Kaplan–Meier plots illustrating patient overall survival outcomes across four time points (T1, T2, T3, and T4), stratifying patients into high and low TCR diversity groups based on three productive rearrangement (panel **a**), Shannon index (panel **b**), and Simpson clonality metrics (panel **c**). The prognostic potential of TCR metrics is most pronounced during the early stages of treatment (T1/T2), with patients exhibiting more polyclonal TCR profiles showing a favorable prognosis

predicting long-term benefit. As we progress to T3 (Cycle 4) and T4 (end of treatment), we observed a diminishing significance and a reduction in the separation of survival outcomes between the high and low TCR diversity groups. Additionally, we performed an examination of the time-to-treatment-failure (TTF) using the same survival analysis method (Supplementary Fig. 2). We did not observe statistically significant differences in TTF between the two patient groups stratified by TCR diversity at the four evaluated time points.

## The global TCR landscape based on TRBV/TRBJ usage

Next, we performed the updated analysis of the variable (V) and joining (J) gene usage patterns across patient subgroups. The process of V(D)J rearrangement forms the foundation of TCR diversity, which is essential for recognizing a broad range of antigens. Figure 3 provides a detailed overview of the gene usage distribution based on the pre-treatment PBMCs of two patient outcome groups (CR/PR vs PD). This figure illustrates the TCR repertoire landscape by displaying the median usage (relative frequency) of V–J pairings, based on an extensive computation of 779 V–J gene combinations across all patients. Compared to the TRBV and TRBJ gene usage distributions, this V–J pairing usage ratio offers a more comprehensive insight into clonal distribution. Overall, there is a consistent V–J pairing landscape when contrasting the two patient groups, but some distinctive patterns emerge when looking into specific gene usage segments.

The top three most abundant TRBV genes identified in this landscape were TRBV20-1, TRBV5-1, and TRBV7-9, while the TRBJ gene usage primarily featured TRBJ2-7 and TRBJ1-1, and TRBJ1-6. Within this spectrum, TRBV20-1/TRBJ2-7 emerged as the most used gene pairs across both groups, with a noticeably higher frequency in the CR/PR group compared to the PD group. This finding is particularly interesting given that two recent studies also reported the usage of the TRBV20-1 segment as a predictive biomarker for patient outcomes in anti-PD-1 treated lung cancer patients and esophageal squamous cell carcinoma patients. Similarly, we noted an elevated relative frequency of TRBV27-1/TRBJ2-7 and TRBV28-1/TRBJ2-7 in the CR/PR group. Another noteworthy observation is the shift of the second most used gene pairs: from TRBV28-1/TRBJ2-7 in the CR/PR group to TRBV5-1/TRBJ2-7 in the PD group. In our previous analysis using survey-resolution assay, we found that TRBJ2-7 was predominantly enriched in CR/PR patients but displayed a more uniform TRBJ distribution in the PD group—a trend that deep sequencing also confirmed. We further performed statistical screening test to prioritize the specific TRBV/TRBJ gene pairings in relation to patient outcomes. Table 1 presents the *p*-values obtained from the Wilcoxon test comparing the PD and CR/PR groups and the Cox regression analysis based on overall survival and progression-free survival. Three gene pairs—TRBV7-2/

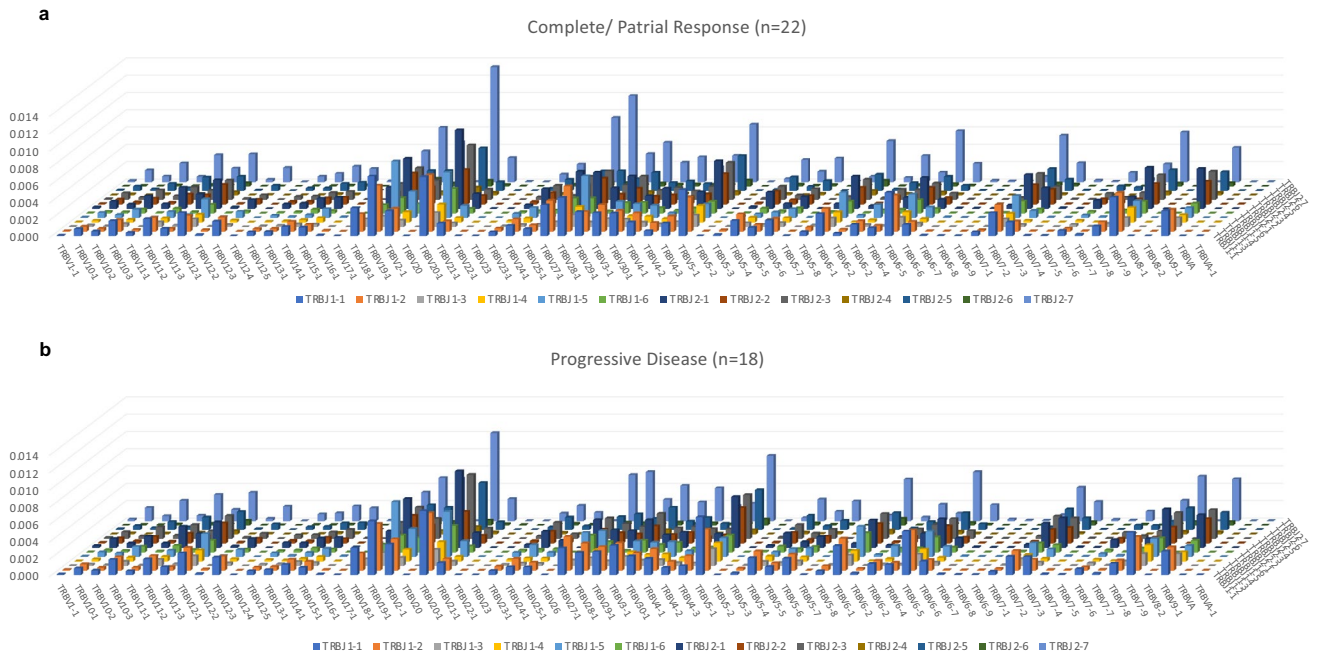


**Fig. 3** The global TCR landscape based on TRBV/TRBJ usage. Overview of V–J pairing distribution in the TCR repertoire landscape based on pre-treatment PBMCs of CR/PR vs. PD patient outcome groups

**Table 1** Table of top TRBV-TRBJ pairs associated with patient outcomes

| TRBV-J | Wilcoxon *p*-value (PD vs CR/PR) | OS *p*-value | PFS *p*-value |
|---|---|---|---|
| TRBV7-2_TRBJ2-1 | 0.004* | 0.774 | 0.229 |
| TRBV6-1_TRBJ1-2 | 0.011* | 0.707 | 0.916 |
| TRBV27-1_TRBJ2-1 | 0.015* | 0.611 | 0.331 |
| TRBV12-1_TRBJ1-2 | 0.020* | 0.688 | 0.492 |
| TRBV28-1_TRBJ2-1 | 0.022* | 0.508 | 0.219 |
| TRBV3-1/03-2_TRBJ2-5 | 0.022* | 0.930 | 0.765 |
| TRBV29-1_TRBJ1-2 | 0.026* | 0.596 | 0.262 |
| TRBV6-2/06-3_TRBJ2-7 | 0.030* | 0.416 | 0.686 |
| **TRBV7-2_TRBJ2-7** | 0.037* | 0.017* | 0.037* |
| TRBV28-1_TRBJ1-1 | 0.037* | 0.256 | 0.791 |
| TRBV28-1_TRBJ2-3 | 0.042* | 0.330 | 0.932 |
| TRBV9-1_TRBJ2-1 | 0.055 | 0.992 | 0.640 |
| **TRBV20-1_TRBJ2-3** | 0.059 | 0.508 | 0.046* |
| TRBV20-1_TRBJ2-7 | 0.062 | 0.668 | 0.602 |
| TRBV2-1_TRBJ1-2 | 0.066 | 0.436 | 0.615 |
| **TRBV7-2_TRBJ1-2** | 0.066 | 0.140 | 0.032* |
| TRBV28-1_TRBJ1-5 | 0.075 | 0.423 | 0.869 |
| TRBV12-1_TRBJ1-5 | 0.095 | 0.505 | 0.808 |
| TRBV2-1_TRBJ2-1 | 0.100 | 0.008 | 0.414 |
| TRBV6-1_TRBJ1-5 | 0.100 | 0.619 | 0.141 |

*p*-values $<0.05$ are denoted with "*"

TRBJ2-7, TRBV20-1/TRBJ2-3, and TRBV7-2/TRBJ1-2—emerged as potential prognostic biomarkers, exhibiting significant or marginally significant associations with both therapy response and survival outcomes.

These results highlight the potential of targeting specific TRBV/TRBJ pairings, especially those with high or intermediate expression, as complementary biomarkers to TCR diversity metrics for enhancing outcome prediction. However, a much larger sample size is needed to validate and translate the prognostic utility of specific gene pairings, given the substantial TCR heterogeneity across individuals and studies.

## Discussion

In this study, we utilized deep-sequencing immunoSEQ analysis to explore the TCR repertoire in PBMC from an expanded cohort of patients with HNSCC enrolled in a phase I/II trial investigating the combination of cetuximab and nivolumab. Given the limited sample size and the intrinsic heterogeneity of HNSCC, we focused on characterizing the dynamics and variability of peripheral TCR profiles across patients and throughout treatment. And we explored their associations with patient outcomes within specific clinical subgroups. Our findings from the updated correlative study corroborate a potential association between baseline peripheral TCR clonality and patient responses to therapy, aligning with observations from our previous analysis using the survey-resolution assay. However, this trend is not uniformly statistically significant across all subgroups, likely due to the sample size limitation and extensive sample heterogeneity. In contrast to non-responders, patients achieving complete or partial responses tended to exhibit a more diverse TCR repertoire, a pattern particularly evident in those with prior immunotherapy, a history of smoking, or HPV-negative tumors. Analysis of TCR metrics across treatment milestones further highlighted the prognostic potential of early TCR diversity: greater polyclonality correlated with favorable long-term patient outcomes. Moreover, the distinct TRBV/TRBJ gene usage profiles we observed across patient response groups, including specific gene pairs associated with survival, suggest their potential as complementary biomarkers for predicting immunotherapy response.

The observed relationship between TCR diversity and patient outcomes in the combination immunotherapy presents important clinical implications. Observations from most studies to date support the notion that a polyclonal TCR repertoire in the peripheral blood, present before the initiation of immunotherapy, is generally advantageous and often associated with better patient outcomes [16]. This notion aligns with the hypothesis that a diverse array of T-cell receptors enhances the immune system's ability to identify a wide range of tumor antigens. However, conflicting reports exist, with studies finding no association or even an inverse pattern [17, 18]. Given the inherent patient heterogeneity and the temporal dynamics of the immune system, the TCR repertoire is subject to fluctuations influenced by various factors, such as prior immune-related treatments, viral infections, and tumor responses. Therefore, characterizing the immune response based on a single snapshot of TCR diversity may not adequately capture its complex and

evolving nature. Recent studies have also highlighted the importance of monitoring peripheral T-cell turnover and TCR dynamics during therapy to better stratify patients in personalized immunotherapy [19, 20]. The survival analysis based on the temporal data suggested that T2 (around C1) demonstrated greater prognostic value compared to pre-treatment TCR profiles. This highlights the importance of continuous immune response monitoring, particularly in the early treatment stages, to enhance outcome prediction.

From a translational perspective, these findings suggest three key applications for optimizing immunotherapy strategies in HNSCC. (1) Upfront patient stratification: Pre-treatment TCR diversity and gene usage patterns could be integrated into predictive models to refine patient selection, potentially guiding combination therapies for those unlikely to respond to standard therapies. (2) Monitoring during treatment: Serial sampling of peripheral TCR diversity at critical early time points could identify patients with favorable immune activation, enabling timely escalation or de-escalation of therapy. This could improve treatment personalization and prevent unnecessary toxicity in non-responders. (3) Post-treatment immune surveillance: longitudinal TCR sequencing may provide useful data on immune memory formation and clonal expansion, which could help assess long-term immunological changes after therapy. Such insights may guide strategies for retreatment or maintenance immunotherapy in recurrent disease, while beyond the scope of this study, future research investigating additional markers, such as blood cell phenotypes [12] as complementary biomarkers, may provide further insights into therapeutic decision-making.

One of the key challenges in interpreting peripheral TCR repertoire data is the intrinsic heterogeneity of TCRs across patients, which is further influenced by clinical and biological factors such as HPV status and smoking history. These factors are known to influence the host immune landscape and may contribute to differences in TCR diversity and clonality observed across patient subgroups. Additionally, the absence of HLA class I data limits our ability to fully assess how antigen presentation dynamics influence TCR clonotype expansion, particularly in the context of checkpoint blockade therapy. Since HLA genotype plays a critical role in determining T-cell recognition of tumor antigens, integrating HLA typing with TCR sequencing could provide deeper insights into whether observed TCR expansions are tumor-specific or reflect broader immune activation.

A major limitation of this study is the absence of matched tumor infiltrating lymphocytes (TILs) data, which prevents a direct comparison between peripheral and intratumoral TCR dynamics. The extent to which peripheral TCR diversity and clonality mirrors the dynamics within the tumor itself remains an open research topic. Emerging studies suggest that circulating tumor-reactive T-cells, though present at low frequencies, exhibit distinct transcriptional profiles compared to their tumor-resident counterparts [21]. Access to tumor-derived TCR sequencing data (e.g., immunoSEQ) could help differentiate T-cell populations that are directly involved in tumor recognition and response from those that are systemically circulating or non-specific. By integrating tumor and peripheral TCR sequencing, researchers might gain deeper insights into how the immune system dynamically adapts to therapy. This, in turn, could enhance our ability to understand both tumor- and treatment-induced immune shifts. However, the extent to which tumor-derived TCR sequences offer a more specific or superior insight remains debatable. Recent research indicates that, following checkpoint inhibitor treatment, the expansion of TILs often arises from novel clonotypes in the peripheral compartment, not pre-existing TILs [22]. This supports an "open compartment" model of the tumor microenvironment, where continuous cross-talk between the tumor and host immune system [19] actively reshapes the TCR landscape. Given the clinical challenges of obtaining tumor biopsies in many settings, we argue that refining tumor-agnostic TCR monitoring models based on less invasive blood samples is both translationally valuable and mechanistically relevant for assessing immune dynamics in response to therapy. We acknowledge that sample availability varied across treatment time points, which may have impacted the statistical power of longitudinal analyses, particularly at later time points. Future studies with larger cohorts and subgroups, as well as more evenly distributed sample collection, will be crucial to further validate these findings.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00262-025-03993-6.

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**Data availability** No datasets were generated or analyzed during the current study.

## Declarations

**Conflict of interest** Conflict of interest: CHC—honoraria from Fulgent, Genmab, AVEO, Seagen, Regeneron, Bicara, Johnson and John-

son, and Exelixis for ad hoc Scientific Advisory Board or steering committee participation. NFS—honoraria from Pfizer, Merck, Aduro, Rakuten, CUE, and Blupoint, Eisai, Astra Zeneca, WebMD, Mirati, Reach MD, Vaccinex, Kura, Biontech, GSK, Aduro, Pfizer for ad hoc Scientific Advisory Board or Data Safety Monitoring Committee and research funding Bristol-Myers Squibb and Exelixis. The other authors do not have conflict of interest to declare.

**Ethics approval and consent to participate** As part of a phase I/II study of concurrent cetuximab and nivolumab in patients with R/M HNSCC (NCT03370276), this study was approved by the Scientific Review Committee, the Institutional Review Board at Moffitt Cancer Center and all participate sites.



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